PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

David N. COOPER et al.

Serial No. (unknown)

Filed herewith

METHOD FOR DETECTING GROWTH
HORMONE VARIATIONS IN HUMANS,
THE VARIATIONS AND THEIR USES

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents

Washington, D.C. 20231

Sir:

Prior to calculation of the filing fee, please amend the above-identified application as follows:

IN THE CLAIMS:

Please amend claim 4 as follows:

--4. (Amended) A method according to claim 1, wherein the individual exhibits normal results in a standard growth hormone function test.--

Please amend claim 5 as follows:

--5. (Amended) A method according to claim 1, wherein the detection method comprises any sequencing method for determining the sequence of the *GH1* gene of an individual.--

Please amend claim 6 as follows:

--6. (Amended) A method according to claim 1, wherein the detection method comprises PCR amplification of the *GH1* gene of the individual using (a) a *GH1* gene-specific fragment, being a fragment unique to the *GH1* gene whose sequence is not found in the four other paralogous (non-*GH1*) genes in the GH cluster, and (b) one or more *GH1* gene-specific primers which cannot bind to the homologous flanking regions in the four other paralogous (non-*GH1*) genes in the GH cluster.

Please amend claim 7 as follows:

--7. (Amended) A method according to claim 1, wherein the detection method comprises PCR amplification of the entire *GH1* gene of the individual and nested PCR of overlapping constituent fragments of the *GH1* gene of the individual. --

Please amend claim 8 as follows:

--8. (Amended) A method according to claim 1, wherein the detection method comprises PCR amplification of all or a fragment of genomic DNA spanning the Locus Control Region of the *GH1* gene.--

Please amend claim 9 as follows:

--9. (Amended) A method according to claim 1, wherein the detection method comprises mutational screening of all or a fragment of the individual's *GH1* gene by DHPLC.--

Please amend claim 11 as follows:

--11. (Amended) A detection method according to claim 10, which detection method further comprises the use of one or more primer(s) selected from:

CTC CGC GTT CAG GTT GGC (GH1DF);

AGG TGA GCT GTC CAC AGG (GH1DR);

GGG CAA CAG TGG GAG AGA AG (GH2DF);

CCT CCA GGG ACC AGG AGC (GH2DR);

CAT GTA AGC CCA GTA TTT GGC C (GH3DF);

CTG AGC TCC TTA GTC TCC TCC TCT (GH3DR);

GAC TTT CCC CCG CTG GGA AA (GH4DF);

GGA GAA GGC ATC CAC TCA CGG (GH4DR);

TCA GAG TCT ATT CCG ACA CCC (GH5DF);

GTG TTT CTC TAA CAC AGC TCT C (GH5DR);

TCC CCA ATC CTG GAG CCC CAC TGA (GH6DF)

CGT AGT TCT TGA GTA GTG CGT CAT CG (GH6DR);

TTC AAG CAG ACC TAC AGC AAG TTC G (GHD7F);

CTT GGT TCC CGA ATA GAC CCC G (GH7DR);

GTGCCCCAAGCCTTTCCC (LCR15: 1159-1177);

TGTCAGATGTTCAGTTCATGG (LCR13: 1391-1412);

CCTCAAGCTGACCTCAGG (LCR25: 1346-1363);

GATCTTGGCCTAGGCCTCG (LCR23: 1584-1602);

LCR 5A (5' CCAAGTACCTCAGATGCAAGG 3');

LCR 3.0 (5' CCTTAGATCTTGGCCTAGGCC 3');

LCR 5.0 (5' CCTGTCACCTGAGGATGGG 3');

LCR 3.1 (5' TGTGTTGCCTGGACCCTG 3');

LCR 3.2 (5' CAGGAGGCCTCACAAGCC 3')

LCR 3.3 (5′ ATGCATCAGGGCAATCGC 3′);
GH1G5 (5′ GGTACCATGGCTACAGGTAAGCGCC 3′);
GH1G3 (5′ CTCGAGCTAGAAGCCACAGCTGCCC 3′);
BGH3 (5′ TAGAAGGCACAGTCGAGG 3′);
GH1R5 (5′ ATGGCTACAGGCTCCCGG 3′); and
GH1R3 (5′ CTAGAAGCCACAGCTGCCC 3′).--

Please amend claim 12 as follows:

--12. (Amended) A variant of *GH1*, which differs from *GH1* and is detected by or is detectable by a method according to claim 1 but was not detected by methods used hitherto, such as those reliant on patient selection criteria based primarily on absolute height.--

Please amend claim 14 as follows:

--14. (Amended) A variant of *GH1* according to claim 12 comprising a missense mutation.--

Please amend claim 15 as follows:

--15. (Amended) A variant of *GH1* according to claim 12 comprising a silent mutation which affects the activity of the signal peptide.--

Please amend claim 17 as follows:

--17. (Amended) A protein or amino acid sequence encoded by a variant of *GH1* according to claim 12.--

Please amend claim 21 as follows:

--21. (Amended) A screening method for screening an individual suspected of GH dysfunction, which screening method comprises the steps of:

(a) obtaining a test sample comprising a nucleotide sequence of the human *GH1* gene from the individual; and

(b) comparing a region of the sequence obtained from the test sample with the corresponding region of a predetermined sequence

wherein the predetermined sequence is selected from a variant of *GH1* according to claim 12.--

Amend claim 24 as follows:

--24. (amended) A screening method according to claim 21, comprising:

(a) obtaining a first test sample from an individual; and

(b) comparing the *GH1* gene or *GH1* transcript, or fragment therefrom (*eg* cDNA), in the first test sample to the corresponding gene, transcript or fragment of a *GH1* variant obtainable from a second test sample derived from an individual exhibiting the following criterion:

(i) growth failure defined as a growth pattern [delineated by a series of height measurements; Brook CDG (Ed) Clinical Paediatric Endocrinology 3rd Ed, Chapter 9, p141 (1995, Blackwell Science)] which, when plotted on a standard height chart [Tanner *et al* Arch. Dis. Child 45 755-762 (1970)], predicts an adult height for the individual which is outside the individual's estimated target adult height range, the

estimate being based upon the heights of the individual's parents.--

Amend claim 26 as follows:

--26. (amended) A screening method according to claim 21 in which simultaneous screens are used either for multiple known mutations or for all possible mutations by hybridization of a labelled sample of DNA (cDNA or genomic DNA derived from the individual) to micro-arrays of mutation-specific oligonucleotide probes immobilised on a solid support.--

Amend claim 28 as follows:

--28. (amended) A kit suitable for use in carrying out a screening method according to claim 21, which kit comprises:

(a) an oligonucleotide having a nucleic acid sequence corresponding to a region of a *GH1* variant, which region incorporates at least one variation from the corresponding wild-type hGH gene sequence; and/or

(b) an oligonucleotide having a nucleic acid sequence corresponding to the wild-type hGH gene sequence in the region specified in (a); and, optionally,

(c) one or more reagents suitable for carrying out PCR for amplifying desired regions of the individual's DNA.--

Cancel claim 29.--

--30. (amended) A kit according to claim 28, wherein kit component (a) comprises a plurality of said oligonucleotides immobilised on a solid support.--

--31. (amended) A kit suitable for use in carrying out a detection method in which the variant is at least one of the variants claimed in claim 12.--

Amend claim 32 as follows:

--32. (amended) A screening method for screening an individual suspected of GH dysfunction, which screening method comprises the steps of:

(a) obtaining a test sample comprising an amino acid sequence encoded by the human *GH1* gene of the individual; and

(b) analysing the test sample for the presence of a GH variant wherein the GH variant is selected from those according to claim 17.--

Amend claim 34 as follows:

--34. (amended) An isolated, purified or recombinant nucleic acid sequence selected from:

(a) a sequence comprising a variant of *GH1* according to claim 12 or

(b) a sequence substantially homologous to or that hybridises to sequence (a) under stringent conditions; or

(c) a sequence substantially homologous to or that hybridizes under stringent conditions to the sequence (a) or (b) but for the degeneracy of the genetic code; or

(d) an oligonucleotide specific for any of the sequences (a), (b) or (c).--

--37. (amended) A process for preparing a variant of *GH1* according to claim 12, which process comprises:
(i) culturing a host cell; and
(ii) recovering from the culture medium the variant of *GH1* thereby produced.--

Amend claim 38 as follows:

--38. (amended) An amino acid sequence encoded or expressed by a sequence, vector, or cell as defined in claim 34 in culture medium.--

Amend claim 39 as follows:

--39. (amended) A composition comprising a variant of *GH1* or a GH variant according to claim 12, respectively, in association with a pharmaceutically acceptable carrier therefor.--

Amend claim 40 as follows:

--40. (amended) Use of a variant of *GH1* or a GH variant according to claim 12, respectively, for a therapeutic, diagnostic or detection method.--

Cancel claim 42.

Cancel claim 43.

Amend claim 44 as follows:

--44. (amended) Use of a variant of GH1 or GH variant according to claim 12, in the preparation of a medicament, diagnostics composition or kit, or detection kit.--

R E M A R K S

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE".

Respectfully submitted,

YOUNG & THOMPSON

By _____

Thomas W. Perkins
Attorney for Applicants
Registration No. 33,027
745 South 23rd Street
Arlington, VA 22202
Telephone: 703/521-2297

May 14, 2001

VERSION WITH MARKINGS TO SHOW CHANGES MADE

4. A method according to ~~any preceding claim,~~ claim 1, wherein the individual exhibits normal results in a standard growth hormone function test.

5. A method according to ~~any preceding claim,~~ claim 1, wherein the detection method comprises any sequencing method for determining the sequence of the *GH1* gene of an individual.

6. A method according to ~~any preceding claim,~~ claim 1, wherein the detection method comprises PCR amplification of the *GH1* gene of the individual using (a) a *GH1* gene-specific fragment, being a fragment unique to the *GH1* gene whose sequence is not found in the four other paralogous (non-*GH1*) genes in the GH cluster, and (b) one or more *GH1* gene-specific primers which cannot bind to the homologous flanking regions in the four other paralogous (non-*GH1*) genes in the GH cluster.

7. A method according to ~~any preceding claim,~~ claim 1, wherein the detection method comprises PCR amplification of the entire *GH1* gene of the individual and nested PCR of overlapping constituent fragments of the *GH1* gene of the individual.

8. A method according to ~~any preceding claim,~~ claim 1, wherein the detection method comprises PCR amplification of all or a fragment of genomic DNA spanning the Locus Control Region of the *GH1* gene.

9. A method according to ~~any preceding claim,~~ claim 1, wherein the detection method comprises mutational screening of all or a fragment of the individual's *GH1* gene by DHPLC.

11. A detection method according to ~~any preceding claim,~~ claim 10, which detection method further comprises the use of one or more primer(s) selected from:
CTC CGC GTT CAG GTT GGC (GH1DF);
AGG TGA GCT GTC CAC AGG (GH1DR);
GGG CAA CAG TGG GAG AGA AG (GH2DF);
CCT CCA GGG ACC AGG AGC (GH2DR);
CAT GTA AGC CCA GTA TTT GGC C (GH3DF);
CTG AGC TCC TTA GTC TCC TCC TCT (GH3DR);
GAC TTT CCC CCG CTG GGA AA (GH4DF);
GGA GAA GGC ATC CAC TCA CGG (GH4DR);
TCA GAG TCT ATT CCG ACA CCC (GH5DF);
GTG TTT CTC TAA CAC AGC TCT C (GH5DR);
TCC CCA ATC CTG GAG CCC CAC TGA (GH6DF)
CGT AGT TCT TGA GTA GTG CGT CAT CG (GH6DR);
TTC AAG CAG ACC TAC AGC AAG TTC G (GHD7F);
CTT GGT TCC CGA ATA GAC CCC G (GH7DR);
GTGCCCCAAGCCTTTCCC (LCR15: 1159-1177);
TGTCAGATGTTCAGTTCATGG (LCR13: 1391-1412);
CCTCAAGCTGACCTCAGG (LCR25: 1346-1363);
GATCTTGGCCTAGGCCTCG (LCR23: 1584-1602);
LCR 5A (5′ CCAAGTACCTCAGATGCAAGG 3′);
LCR 3.0 (5′ CCTTAGATCTTGGCCTAGGCC 3′);
LCR 5.0 (5′ CCTGTCACCTGAGGATGGG 3′);
LCR 3.1 (5′ TGTGTTGCCTGGACCCTG 3′);
LCR 3.2 (5′ CAGGAGGCCTCACAAGCC 3′);
LCR 3.3 (5′ ATGCATCAGGGCAATCGC 3′);
GH1G5 (5′ GGTACCATGGCTACAGGTAAGCGCC 3′);
GH1G3 (5′ CTCGAGCTAGAAGCCACAGCTGCCC 3′);
BGH3 (5′ TAGAAGGCACAGTCGAGG 3′);
GH1R5 (5′ ATGGCTACAGGCTCCCGG 3′); and
GH1R3 (5′ CTAGAAGCCACAGCTGCCC 3′).

12. A variant of *GH1*, which differs from *GH1* and is detected by or is detectable by a method according to~~any preceding~~ claim 1 but was not detected by methods used

hitherto, such as those reliant on patient selection criteria based primarily on absolute height.

14. A variant of *GH1* according to ~~any preceding~~ claim 12 comprising a missense mutation.

15. A variant of *GH1* according to ~~any preceding~~ claim 12 comprising a silent mutation which affects the activity of the signal peptide.

17. A protein or amino acid sequence encoded by a variant of *GH1* according to any of ~~claims 12 to 16.~~ claim 12.

21. A screening method for screening an individual suspected of GH dysfunction, which screening method comprises the steps of:
(a) obtaining a test sample comprising a nucleotide sequence of the human *GH1* gene from the individual; and
(b) comparing a region of the sequence obtained from the test sample with the corresponding region of a predetermined sequence
wherein the predetermined sequence is selected from a variant of *GH1* according to ~~any of claims 12 to 16~~ 12.

24. A screening method according to ~~any one of claims 21 to 23,~~ claim 21, comprising:
(a) obtaining a first test sample from an individual; and
(b) comparing the *GH1* gene or *GH1* transcript, or fragment therefrom (*eg* cDNA), in the first test sample to the corresponding gene, transcript or fragment of a *GH1* variant

obtainable from a second test sample derived from an individual exhibiting the following criterion:
(i) growth failure defined as a growth pattern [delineated by a series of height measurements; Brook CDG (Ed) Clinical Paediatric Endocrinology 3rd Ed, Chapter 9, p141 (1995, Blackwell Science)] which, when plotted on a standard height chart [Tanner *et al* Arch. Dis. Child 45 755-762 (1970)], predicts an adult height for the individual which is outside the individual's estimated target adult height range, the estimate being based upon the heights of the individual's parents.

26. A screening method according to ~~any of claims~~ claim 21~~to 25~~ in which simultaneous screens are used either for multiple known mutations or for all possible mutations by hybridization of a labelled sample of DNA (cDNA or genomic DNA derived from the individual) to micro-arrays of mutation-specific oligonucleotide probes immobilised on a solid support.

28. A kit suitable for use in carrying out a screening method according to~~any of claims~~ claim 21~~ to 27~~, which kit comprises:
(a) an oligonucleotide having a nucleic acid sequence corresponding to a region of a *GH1* variant, which region

incorporates at least one variation from the corresponding wild-type hGH gene sequence; and/or

(b) an oligonucleotide having a nucleic acid sequence corresponding to the wild-type hGH gene sequence in the region specified in (a); and, optionally,

(c) one or more reagents suitable for carrying out PCR for amplifying desired regions of the individual's DNA.

30. A kit according to claim 28 ~~or claim 29~~, wherein kit component (a) comprises a plurality of said oligonucleotides immobilised on a solid support.

31. A kit suitable for use in carrying out a detection method in which the variant is at least one of the variants claimed in ~~claims 12 to 16~~ claim 12.

32. A screening method for screening an individual suspected of GH dysfunction, which screening method comprises the steps of:

(a) obtaining a test sample comprising an amino acid sequence encoded by the human *GH1* gene of the individual; and

(b) analysing the test sample for the presence of a GH variant wherein the GH variant is selected from those according to ~~any one of claims 17 to 20~~ claim 17.

34. An isolated, purified or recombinant nucleic acid sequence selected from:

(a) a sequence comprising a variant of *GH1* according to ~~any of claims 12 to 16 or encoding a GH variant according to any of claims 17 to 20~~ claim 12 or

(b) a sequence substantially homologous to or that hybridises to sequence (a) under stringent conditions; or

(c) a sequence substantially homologous to or that hybridizes under stringent conditions to the sequence (a) or (b) but for the degeneracy of the genetic code; or

(d) an oligonucleotide specific for any of the sequences (a), (b) or (c).

37. A process for preparing a variant of *GH1* according to ~~any of claims 12 to 16~~ claim 12, which process comprises:

(i) culturing a host cell ~~according to claim 36~~; and

(ii) recovering from the culture medium the variant of *GH1* thereby produced.

38. An amino acid sequence encoded or expressed by a sequence, vector, or cell as defined in ~~any of claims~~ claim 34~~to 37~~ in culture medium.

39. A composition comprising a variant of *GH1* or a GH variant according to ~~any of claims 12 to 16 or 17 to 20,~~ claim 12, respectively, in association with a pharmaceutically acceptable carrier therefor.

40. Use of a variant of *GH1* or a GH variant according to ~~any of claims 12 to 16 or 17 to 20,~~ claim 12, respectively, for a therapeutic, diagnostic or detection method.

44. Use of a variant of GH1 or GH variant according to ~~any of claims 12 to 16 or 17 to 20 respectively,~~ claim 12, in the preparation of a medicament, diagnostics composition or kit, or detection kit.